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Attorney's Docket No. 8151-24A

PATENT

8-01-01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re:	Hanley, Jr. et al.	
Appl. No.:	09/560,288	Group Art Unit: 1633
Filed:	April 27, 2000	Examiner: Kerr, J.
For:	METHOD FOR PRODUCING HUMAN INTERVERTEBRAL DISC CELLS	

July 18, 2001

DECLARATION UNDER RULE 132 OF DR. HELEN GRUBER

I, Dr. Helen E. Gruber, declare and state:

1. That I received my B.S. degree in pre-medicine from the University of Idaho in 1969, my M.S. degree in cell biology, radiation biology and microbiology from Oregon State University in 1974, and my doctorate in cell biology from Oregon State University in 1976. I am presently a faculty member, interdisciplinary PhD program, Department of Biology, at the University of North Carolina at Charlotte, and adjunct professor, Greenville Hospital System/Clemson University Biomedical Cooperative. Also, I have 100 refereed scientific articles in print and have co-authored 11 book chapters. A copy of my CV appears as Exhibit A;

2. That I am a co-inventor of United States patent application Serial No. 09/560,288 filed April 27, 2000 entitled *Method for Producing Human Intervertebral Disc Cells*.

3. That I have read the Office Acton dated May 9, 2000 and have considered the examiner's positions that "the claims are not enabled as the specification does not provide sufficient guidance for one skilled in the art to provide the claimed composition which is therapeutically effective in treating human disc diseases."

This Declaration responds to the issues raised by the examiner.

4. The state of the art is such that an orthopedic spine surgeon can perform the procedure set forth in the claims. The surgeon would first remove healthy disc tissue from a disc that does not show degenerative changes using an open procedure or a percutaneous procedure. The patient's own disc cells would be grown in tissue culture as described in the patent specification. These cells would then be provided to the surgeon who would place them into a debrided segment of degenerating disc. Over time these cells and their descendents would form appropriate building blocks of disc tissue in the implanted site. A schematic illustration of this procedure is shown in Figure 1 of the patent application.

5. The sand rat (*Psammonys obesus*) is routinely used as a model for studying autologous disc cell implantation because it spontaneously develops disc degeneration during aging, like humans do. Thus, the sand rat model is the closest animal to the human for these studies. The sand rat is a model accepted by researchers doing disc studies and the results are translatable to human. There is, in my opinion, no reason for not accepting the results of disc studies performed on sand rats as being applicable to humans.

6. The first issue is to the type of disc cells that are cultured to make the therapeutic composition claimed by this invention. The human cells that would be cultured *in vitro* are cells from the annulus. The type of human cells obtained from expansion of disc tissue *in vitro* which are used in the claimed therapeutic composition are cells from the annulus. There is detailed discussion and examples (Note examples 4 and 5, for instance) of the use of annulus cells in the patent application. In my opinion, the patent application provides sufficient guidance for one skilled in the art to provide the claimed composition using disc cells from the annulus.

7. Next the patent examiner questions whether the cells in the therapeutic composition are de-differentiated, proliferating cells, or whether the cells are differentiated and secreting an extra cellular matrix that is representative of the matrix observed in a normal intervertebral disc *in vivo*. The state of differentiation would be either de-differentiated (flattened cells grown in monolayer on traditional plastic cultureware) or differentiated (rounded cells) grown in three-dimensional microenvironments such as alginate, agarose, or collagen sponges.

8. The annulus cells are proliferating cells. To show this, I have conducted studies that show the annulus cells of the therapeutic composition are proliferative. Cells were harvested from a singular lumber vertebral disc site of a sand rat. Consistent with the disclosure of the patent application, cells were cultured through primary explant at which time they were labeled in monolayer culture, rinsed in buffer, trypsinized, centrifuged, resuspended in 9 1 buffer, and inserted into a 2x2 mm section of Gelfoam® presoaked in Minimal Essential Media. Attached as Exhibit B are photomicrographs of the sand rat disc cells cultured from the annulus disc cells. Figure A shows attachment. Figure B shows proliferation of cells. A mitotic figure is shown. (A, B: paraffin embedded, H&E, X 640).

9. In addition, my studies show that the cultured annulus disc cells in the therapeutic composition are secreting extracellular matrix components including type I collagen, type II collagen, chondroitin sulfate and keratin sulfate. Using the cells cultured in the experiment of paragraph 8, photomicrographs were made. The results are shown in Figures C-F of Exhibit B, wherein secretion of extracellular matrix components can be seen. Figures C-F show immunolocalization of extracellular matrix products following 10 days of culture of sand rat annulus cells on the collagen carrier. The immunolocalization areas are localized with a purple-black color. C, keratin sulfate; D, chondroitin sulfate; E, Type II collagen; F, Type I collagen. G is a representative negative control showing no localization in the cluster of cells marked by the arrows. (C-G paraffin embedded, X 295).

10. Having shown and claimed the use of annulus cells in the therapeutic compositions of this invention, it is my opinion that it would not require undue experimentation to make and use the invention as claimed.

11. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that

these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Helen E Gruber
Dr. Helen E. Gruber

July 18, 2001
Date

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